

## CLAIMS

We claim:

1. An array composition comprising:

a) a substrate with a surface comprising discrete sites; and  
b) a population of microspheres comprising at least a first and a second subpopulation,  
wherein each subpopulation comprises:

i) a bioactive agent; and  
ii) an identifier binding ligand that will bind a decoder binding ligand such that the  
identification of the bioactive agent can be elucidated;

wherein said microspheres are distributed on said surface.

2. An array composition comprising:

a) a substrate with a surface comprising discrete sites; and  
b) a population of microspheres comprising at least a first and a second subpopulation,  
wherein each subpopulation comprises a bioactive agent and does not comprise an optical  
signature, wherein said microspheres are distributed on said surface.

3. A composition according to claim 1 or 2 further comprising at least one decoder binding ligand.

4. A composition according to claim 1 or 2 wherein said bioactive agents are nucleic acids.

5. A composition according to claim 1 or 2 wherein said bioactive agents are proteins.

6. A method of making a composition comprising:

a) forming a surface comprising individual sites on a substrate;  
b) distributing microspheres on said surface such that said individual sites contain  
microspheres, wherein said microspheres comprise at least a first and a second  
subpopulation each comprising a bioactive agent and do not comprise an optical signature.

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7. A method of making a composition comprising:

- a) forming a surface comprising individual sites on a substrate;
- b) distributing microspheres on said surface such that said individual sites contain microspheres, wherein said microspheres comprise at least a first and a second subpopulations each comprising:
  - i) a bioactive agent; and
  - ii) an identifier binding ligand that will bind at least one decoder binding ligand such that the identification of the bioactive agent can be elucidated.

8. A method of decoding an array composition comprising

- a) providing an array composition comprising:
  - i) a substrate with a surface comprising discrete sites; and
  - ii) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent; wherein said microspheres are distributed on said surface;
- b) adding a plurality of decoding binding ligands to said array composition to identify the location of at least a plurality of the bioactive agents.

9. A method according to claim 8 wherein at least one subpopulation of microspheres comprises an identifier binding ligand to which a decoding binding ligand can bind.

10. A method according to claim 8 wherein said decoding binding ligands bind to said bioactive agents.

11. A method according to claim 8 wherein said decoding binding ligands are labeled.

12. A method according to claim 8 wherein the location of each subpopulation is determined.

13. A method of determining the presence of a target analyte in a sample comprising:

- a) contacting said sample with a composition comprising:

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- i) a substrate with a surface comprising discrete sites; and
  - ii) a population of microspheres comprising at least a first and a second subpopulation each comprising a bioactive agent and do not comprise an optical signature; wherein said microspheres are distributed on said surface such that said discrete sites contain microspheres; and
- b) determining the presence or absence of said target analyte.

14. A method of determining the presence of a target analyte in a sample comprising:

a) contacting said sample with a composition comprising:

- i) a substrate with a surface comprising discrete sites; and
  - ii) a population of microspheres comprising at least a first and a second subpopulation each comprising:
    - 1) a bioactive agent; and
    - 2) an identifier binding ligand that will bind a decoder binding ligand such that the identification of the bioactive agent can be elucidated;wherein said microspheres are distributed on said surface such that said discrete sites contain microspheres; and
- b) determining the presence or absence of said target analyte.

15. An array composition comprising:

- a) a substrate with a surface comprising discrete sites;
- b) a population of microspheres distributed on said sites and comprising at least a first and a second subpopulation, each subpopulation comprising a bioactive agent, wherein said first subpopulation comprises at least a first optical dye with a first pKa and said second subpopulation comprises at least a second optical dye with a second pKa, wherein said first and second pKas are different.

16. A method of making a microsphere array comprising:

- a) contacting a substrate with a surface comprising discrete sites with a solution comprising a population of particles; and

b) applying energy to said substrate or said solution, or both, such that at least a subpopulation of said particles associate onto sites.

17. A method according to claim 16 wherein said discrete sites comprise wells.

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18. A method according to claim 16 wherein said energy is in the form of agitation.

19. A method comprising:

a) providing an array composition comprising a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises:

i) a bioactive agent; and

ii) at least a first and a second decoding attribute; and

b) detecting each of said first and second decoding attributes to identify each of said bioactive agents.

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20. A method according to claim 19, wherein said first decoding attribute comprises an identifier binding ligand.

21. A method according to claim 20 wherein said identifier binding ligand comprises a nucleic acid.

22. A method according to claim 19 wherein said second decoding attribute is selected from the group consisting of a second identifier binding ligand, microsphere size or microsphere surface texture.

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23. A method according to claim 19 wherein said first and second attributes are IBLs and are attached to said first subpopulation of microspheres at a first ratio and are attached to said second population of microspheres at a second ratio.

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24. A method according to claim 19 wherein said subpopulations are distributed on a substrate.

25. A method according to claim 19 wherein said array is a liquid array.

26. A method according to claim 25, wherein said detecting is by FACS.

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